STUDY OF POST SHELF LIFE AND MARKETED TABLETS OF DIETHYLCARBAMAZINE CITRATE BY RP-HPLC

[1]Prachi Santosh Dofe, [2]Silesh J. Wadher, [3]Dhanshree Bodke, [4]Shubhada Lakhmale

[1]Student, [2]Professor, [3]Student, [4]Student
[1]Quality Assurance
[1]Swami Ramanand Teerth Marathwada University, Nanded, India

Abstract: To develop and validate a simple, sensitive, and accurate RP-HPLC method for quantifying diethylcarbamazine citrate (DEC) in expired and non-expired tablets. Experiment was run with mobile phase at pH 4.9 using ODS C18 Column (150mm×4.6mm,5umparticle size) L6 6AD pump in which sample is injected at ambient temperature for 10 min run time with flow rate 1ml/min and detection were identified at 210nm by UV detector. The sample retention time is 2.365 min The initial dissolution test was carried out on Electro lab dissolution tester at $37^0C \pm 0.5^0C$. Result of this investigation showed that the drug content didn't change significantly. The % assay was found to be of non-expired tablet was 98% and expired tablet was 78.48%.

Index Terms **- Diethylcarbamazine Citrate, Dissolution, Non-Expired Tablet, Expired Tablet, Assay, RP-HPLC**

INTRODUCTION

Diethylcarbamazine citrate (DEC), chemically designated as N, N-dimethyl-4-methylpiperazine-1-carboxamide dihydrogen citrate, is a piperazine-derived anthelmintic agent indicated for the treatment of lymphatic filariasis, tropical pulmonary eosinophilia, and loiasis. In contrast to previous studies, we modified the mobile phase composition, employing buffer and acetonitrile in a 60:40 (v/v) ratio with a flow rate of 1 mL/min. Given the considerable wastage of medications due to underutilization, reverse-phase high-performance liquid chromatography (RP-HPLC) analysis of post-shelf-life and commercially available DEC tablets is imperative. Evidence suggests that even 15 years beyond their labeled expiration date, approximately 90% of pharmaceuticals—both prescription and over-the-counter—retain high quality. Consequently, expiration dates do not represent a definitive point at which a drug loses potency or becomes unsafe. Method validation, therefore, is essential to confirm the suitability of an analytical procedure for its intended purpose. Evaluating validation outcomes enables assessment of the reliability, reproducibility, and precision of the analytical results. Pharmaceutical analysis encompasses the determination of identity, strength, characterization, and purity of drugs and chemicals. Investigations into the quality of expired products have been limited, and studies on the long-term stability of dosage forms remain relatively scarce.

NEED OF THE STUDY.

Develop and validate a simple, sensitive, and robust RP-HPLC method for DEC quantification in compliance with ICH Q2(R1) guidelines. To compare the drug content, dissolution profiles, and physical properties (hardness, disintegration) of expired and non-expired DEC tablets. The purpose of this study was to provide an HPLC method for the estimation of diethylcarbamazine citrate in pharmaceutical formulations that was quick, simple, accurate, precise, reliable, and least time-consuming. The created method has been verified in accordance with ICH requirements, and it has been advised that the method's specificity, linearity, precision, accuracy, and ruggedness be achieved. The tablet content can be estimated with good linearity, sensitivity, accuracy, and precision using the suggested RP-HPLC method. The creation of a selective and validated RP-HPLC method for diethylcarbamazine citrate which has the potential to separate the medication, is reflected in this study. The dissolving profiles of expired and non-expired diethylcarbamazine citrate tablets varied, although there were no physical differences between them according to this pilot investigation. It is anticipated that expired diethylcarbamazine citrate tablets will be less effective since they released less energy than non-expired tablets. Additionally, it was discovered that the percentage assay of expired tablets was lower than that of non-expired tablets. The developed RP-HPLC method can be used for the suitable quality control test for the estimation of diethylcarbamazine citrate (DEC) in bulk, marketed tablets and expired tablet.

MATERIALS AND METHODS:

Chemicals and reagent:

The Diethylcarbamazine Citrate (API) reference sample was acquired from Yarrow Chem Drugs and Pharmaceuticals (Mumbai). The commercial formulation of Diethylcarbamazine Citrate 150mg was obtained from a local medical supplier, while acetonitrile and potassium dihydrogen orthophosphate buffer were obtained from Research Lab Finchem Industries in Mumbai.

Instruments:

1. UV-visible Spectrophotometer (Shimadzu Model), Model Shimadzu Limited 1800240 UV
2. HPLC: model Shimadzu DGU20 A5R, C18 column ODSC18(150mm×4.6mm,5umparticle size) UV detector, equipped with a solvent delivery pump, sample injector and column thermostat. Lab solution software was applied for data collecting and processing
3. Ultra sonic Bath -Athena Technology
4. Digital pH. meter: Systonic CS
5. HPLC grade water system Research lab fine chem industries, Mumbai
6. Analytical weighing balance: A named; Model AA-2200. [Max,200g, Min 0.01g; e=0.0001].

Instrumentation and Chromatographic Conditions:

This study used a Peak HPLC system equipped with a L6 6AD pump, a variable wavelength programmable using UV-Visible detector, and a Rhonyde injector. The chromatographic analysis was conducted using an Oyster ODS 3,150-4.6mm, 5µm column. The mobile phase was degassed using an ultrasonic bath sonicator. Materials has been weighed using a Digital Analytical balance (Citizen/2017/16305424). Experiment has been conducted with a mobile phase consisting of a mixture of pH 4.9 and ACN (60:40) v/v at pH 4.9 using an Oyster ODS column with a diameter of 150mm×4.6mm, and a thickness of 5µm. A 25µl sample was injected at room temperature for ten minutes at a flow rate of 1.0 ml/min. The maximum wavelength was detected at a wavelength of 210 nm. The duration of sample retention was 2.365 minutes.

Selection of mobile phase:

A range of solvents with varying compositions were tested to determine the optimal mobile phase. The ultimate optimized state was established by assessing the impact of three variables. A-Mobile Phase, B-Flow rate, C-Wavelength Modulation. In consideration of retention time, the mobile phase was chosen as the optimal condition. Buffer: Acetonitrile (60:40, volume/volume) at a flow rate of 1 milliliter per minute at a wavelength of 210 nanometers.

Preparation of standard stock solution:

A precise weight of 2.5 milligrams of drug standard was measured and transferred. A volume of 25 ml of acetonitrile was introduced, subjected for sonication to dissolve, and followed by dilution with diluents by shaking and sonicating. A 0.45-micron membrane filter was employed for filtration.

Analysis of marketed formulation:

A total 20 commercially available tablets of diethylcarbamazine citrate were weighed, thoroughly crushed, and subsequently finely powdered. A precisely measured quantity of powder containing 100mg of diethylcarbamazine citrate was introduced into a 100 ml volumetric flask. The volume was adjusted using the mobile phase, and the contents were stirred for five minutes before being subjected to sonication for dissolution. The dissolution media were prepared by dissolving 2 gm of sodium chloride in 7 ml of hydrochloric acid) 900 ml of gastric buffer with pH 1.3 prepared. A 100-parts per million (ppm) solution was prepared from the powder by addition of the mobile phase, and then passed through a 0.45-micron membrane filter. An exemplary chromatogram of diethylcarbamazine citrate is depicted in Figure 1.

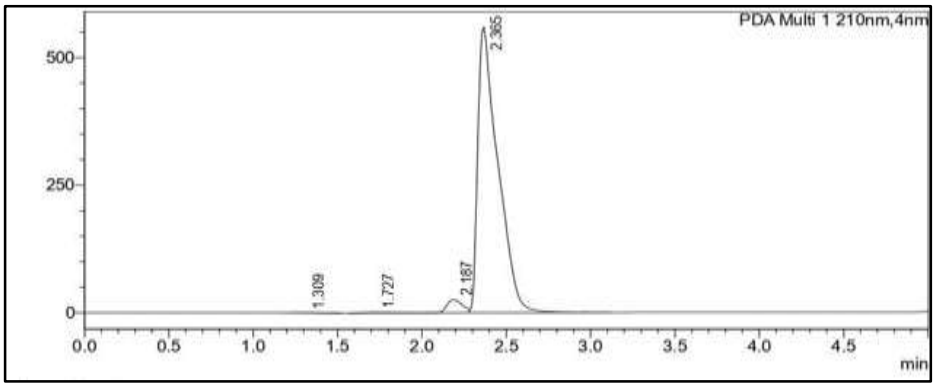


Fig. 1: A typical chromatogram of diethylcarbamazine citrate

Experimental Method:

Selection of wavelength by UV-Visible Spectrophotometry-
In this section the process for selecting the appropriate wavelength was done by using UV-Visible spectrophotometry.

Method of UV Determination:
The UV spectrum of diethylcarbamazine citrate (DEC) was recorded using UV spectrophotometer to determine λ max the 25 µg/ml sample of DEC was prepared and analyzed on UV 200-400 nm to obtain the spectrum.

Preparation of standard stock solution of Diethylcarbamazine citrate-
Accurately weighed 2.5 mg of drug was added and transfer into 50ml volumetric flask 25ml of acetonitrile sonicated and further diluted up to the mark with diluents to get concentration 25µg/ml.

Determination of Maximum wavelength-
Standard solution was scanned separately between 200-400 nm using acetonitrile as blank and the max wavelength 210 nm selected for estimation of drug. The UV spectrum is shown in fig.2

Fig.2 UV Spectrophotometer of standard stock solution

Preparation of phosphate buffer:
Accurately weighed 6.78 g of potassium dihydrogen phosphate were added to 1000 ml of water, and the pH was adjusted with diluted orthophosphoric acid to make it ± 2. Degas it and filtered using a 0.45 µ nylon membrane disc filter.

Selection of Chromatographic Method:
Proper selection of the method depends upon the nature of sample. The drug selected in the present study is polar in nature so in normal phase chromatography method is used. Here the reverse phase HPLC method was selected for the initial separation owing to its simplicity suitability ruggedness and its wider use.

Selection of Mobile Phase.
Working standard solution of diethylcarbamazine citrate were prepared and injected into HPLC system and run in different individual solvent as well as combination of solvent system. Different mobile phase like acetonitrile methanol, acetonitrile water, sodium phosphate buffer: acetonitrile: methanol, different buffers with different pH were tried in order to find the optimum conditions for the good stable peak of Diethylcarbamazine Citrate. Finally mobile phase containing (60:40: v/v) buffer: acetonitrile was selected, since it gives sharp peaks with symmetry within limits and significant retention times for the drug DEC.

Chromatographic conditions:

Table 1: Chromatographic condition

Column	ODS C18 Column (150×4.6mm,5um particlesize)
Flow rate	1ml/min
Injection volume	20ul/ml
Wavelength	210nm
Column temperature	30^0c
Auto sampler tempt.	25^0c
Run time	8 min
Retention time	2.36
Mobile phase	Buffer Acetonitrile 60:40v/v

Method Validation

Specificity:

The capacity to accurately evaluate the analyte in the presence of potentially predicted components is known as specificity. Usually, these could consist of matrix, degradants, etc. When it comes to assays, specificity needs to be demonstrated by proving that the excipients have no effect on the process. The capacity of an analytical procedure to measure analyte of interest precisely and without interference from blank and placebo was known as specificity.

Linearity and Range:

Preparation of linearity sample:

Aliquots of 0.5,1.0,1.5,2.0,2.5, and 3.0 ml were transferred from the DEC stock solution into aseries of 10 ml volumetric flasks, and the volume was adjusted with Acetonitrile. Chromatograms were recorded after five replicates of each concentration were injected. A calibration curve was created by plotting the peak drug area against the drug concentration afterthe peak drug area was measured. A linear response was noted in the 5-30. Within the previously mentioned concentration range, there is an excellent link between the drug's peak area and concentration. Preparation of sample solution for linearity given in table 2.

Table 2: Preparation of sample solution for linearity

ml of stock taken	Diluted to (ml)	Concentration ug/ml
5from 10ug/ml	10	5ug/ml
1	10	10ug/ml
1.5	10	15ug/ml
2	10	20ug/ml
2.5	10	25ug/ml

Precision:

Intra-day and Inter-day Precision:

One set of three distinct standard solution concentrations was made for the intraday fluctuation's studies. To document any intraday fluctuations in the results, each solution was examined three times in the same day. Three distinct days and three distinct concentrations were used for the analysis for the interday variability research. Six solutions with varying concentrations were made each day, and readings were recorded.

Accuracy:

In order to conduct recovery studies, the procedure was applied to the sample's drug content, to which a known quantity of standard was added at 80%, 100%, and 120% levels. The methodentails mixing preanalyzed sample solution with standard drug solution. After injecting the resultant sample solutions, a chromatogram was recorded. The calibration curve was used to calculate the drug's concentration. Three assessments were carried out at every stage.

$$\text{Amount Recovered} = \frac{\text{Amount found}}{\text{Amount Added}} \times 100$$

$$\text{Amount Added} = \text{Weight of API} \frac{\text{Purity of API}}{100}$$

$$\text{Amount found} = \frac{\text{Test Response}}{\text{Standard Response}} \times \frac{\text{Std.Wt.}}{\text{Std.Dilution}} \times \frac{\text{Test dilution}}{1} \times \frac{\text{Standard purity}}{100}$$

Robustness:

Analytical method robustness was tested to ensure that acetonitrile was not impacted by intentional, modest changes in technique parameters. This test also shows how reliable the method is under typical operating conditions. Six injections of the standard solution (10 ppm)were made under various flow conditions, and chromatograms and wavelength readings were taken. Condition for robustness is given in table 3.

Table 3: Condition for Robustness

Condition	Wavelength
Original wavelength	210nm
Increased wavelength	212nm
Decreased wavelength	208nm
Original Flow	1ml/min
Increased Flow	1.2ml/min
Decreased Flow	0.8ml/min

System suitability parameter:

System suitability test are an integral part of gas and liquid chromatographic method. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The test is based on concept operation and sample to be analyzed constitute an integral system that can be evaluated as such. It is the verification of the system to ensure system performance before or during the analysis. Parameter such as plate count, tailing factor, reproducibility and resolution are determined and compared against the specification set for the method. The area under curve (AUC)of five replicate injections should not be more than 2% of relative standard deviation (RSD).

Table 4: System Suitability Parameter

Tailing factor	1.2
Theoretical plates	1546
Retention time	2.365
Pressure	97
Area	4639120

Results and Discussion:

Results of linearity:

Preparation of calibration curve Linearity stock solution preparation:
Accurately weighed quantity 2.5mg DEC was dissolved in mobile phase and volume was made to 25 ml. The standard stock solution of DEC was diluted appropriately to get series of concentrations 20-100 µg/ml for DEC 100 µg/ml. The curve is shown in below figure 4 and results are given in table 5.

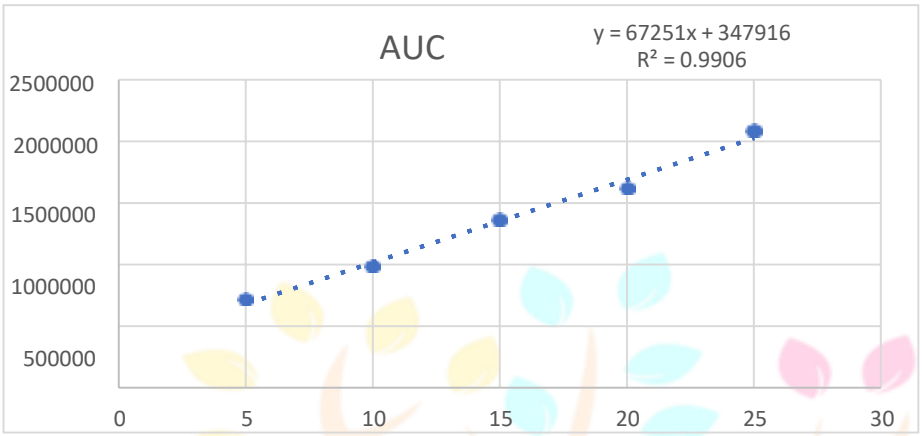


Fig 4: Calibration curve for linearity

Table 5: Concentration with peak areas for linearity

Sr. No.	Diethylcarbamazine citrate conc. (µg/ml)	Peak Area
1	5 µg/ml	1189512
2	10 µg/ml	989127
3	15 µg/ml	1364836
4	20 µg/ml	1621793
5	25 µg/ml	2086279

Accuracy:

80%Recovery-
To carry out recovery research at 80%, 120mg of DEC API total of 147.18 mg of DEC were added to the table powder. Once the mixture had been sufficiently pulverized, the powder was gathered and transferred into a 100 ml volumetric flask. After shaking for 15 minutes, the contents were filtered using microfilter paper No. 0.45. A concentration solution containing 25µg/ml DEC was prepared by serial dilution. The peak area was analyzed by injecting around 25 µl of the sample solution.

100%Recovery:
To achieve complete recovery, 15 mg was included into the tablet powder, which was weighted at 27.18 mg, or 100 mg of DEC. After the mixture had been fully triturated, the powder was collected and placed into a 100 ml volumetric flask. After approximately 15 minutes of shaking, the contents were filtered using microfilter paper No. 45. A solution with a concentration of 10 µg/ml of DEC was prepared by the serial dilution algorithm. To identify the peak locations, around 10 µl of the sample solution were injected.

120%Recovery:
For the recovery research at 120%, a total of 27.18 mg of tablet powder, equivalent to 100 mg of DEC, was weighed. Subsequently, 18 mg of DEC was added to this quantity. Following complete turbulence of the mixture, the powder was gathered and transferred into a 100 ml volumetric flask. After 15 minutes of agitation, the contents were carefully filtered using microfilter paper No.45.Using the serial dilution approach, a concentration solution containing 10 µg/ml of DEC was prepared. To identify the peak areas, 10µl of the sample solution were injected.

Table 6: Accuracy of diethylcarbamazine citrate

Level	Amount of diethylcarbamazine citrate added (ppm)	Amount recovered	% Recovery
80%	120	117.6	98%
80%	120	117.6	98%
80%	120	117.6	98%
100%	150	147	99%
100%	150	147	99%
100%	150	147	99%
120%	180	176.4	98%
120%	180	176.4	98%
120%	180	176.4	98%

Table 7: Recovery data for diethylcarbamazine citrate

METHOD	Level of Recovery	Drug	Mean %Recovery	Standard Deviation	%RSD
HPLC Method	80%	DEC	99.80	1.85	0.035
	100%		100.13	1.24	1.833
	120%		100.12	1.74	1.008

Precision:
Standard deviation and relative standard deviation are often employed to quantify the accuracy of an analytical procedure. Multiple metrics, including repeatability and intermediate precision (intra-day and inter-day), were employed to compute the precision. To validate repeatability, three analyses of DEC 100 µg/ml were performed. An identical concentration of solution was examined three times throughout the day to compute the intraday precision, while the interday precision was determined over a period of three days.

Table 8: Precision of diethylcarbamazine citrate non-expired tablet and expired tablet

Concentration	Injection	Peak area of non-expired tablet	% Assay	Peak area of expired tablet	% Assay
Concentration 100ppm	1	937103	92.52	487889	78.48
	2	947246	93.6	474225	76.32
	3	924675	91.44	480605	77.04
	4	925678	91.44	495678	79.56
	5	932456	92.16	482456	77.40
	6	947865	93.60	472347	75.96
	Mean	935837.166	92.46	336866.66	77.46
	SD	10155.46	0.977	8686.92	1.35
	%RSD	1.09%	1.057	1.802	1.749%

Robustness:

The robustness of the analytical method was assessed by measuring its capacity to maintain stability even with slight fluctuations in method parameters, such as a change in flow of ± 0.2 millilitres per minute or a change in wavelength of ± 2 nanometres.

Table 9: Robustness Parameter

Chromatographic factor for HPLC	Units	% RSD	% Assay
Flowrate	0.9mL	0.32%	94.56
	1.1mL	0.19%	93.87
Wavelength	208	0.181%	94.44
	212	0.134%	95.87

Limit of detection (LOD) and Limit of quantification (LOQ):
The lowest analyte concentration in a sample that can be identified but not always quantified is known as LOD. The lowest analyte concentration in a sample that can be accurately and precisely quantified is known as the limit of quantification, or LOQ.
LOD and LOQ was calculated by using following formulae- LOD = 3.3 σ/slope, LOQ = 10 σ/slope LOD=3.3×1.98÷6725 LOD=0.0097(Where σ= the standard deviation of the response and S=slope of calibration curve)

LOQ=10×1.98÷67250.68=0.0029.

Dissolution Test:

The initial dissolution tests were conducted using an electrical laboratory dissolution tester configured with an IP Apparatus II at 37oC±0.5°C. Each dissolution test was conducted at a medium speed of 50 rpm for 30 minutes in a solution media consisting of 900 ml of gastric buffer pH 1.3 produced by dissolving 2 gm of sodium chloride in 7 ml of hydrochloric acid) and 500 ml of water for dilution with 1000 ml of water. A tablet of diethylcarbamazine citrate was placed in one bowl and expired diethylcarbamazine citrate 150 was added to another bowl. The spinning speed of 50 revolutions per minute was set for 30 minutes. 5 milliliters were withdrawn from both media and the volume was adjusted to the mark to make a standard with the same concentration. The resulting mixture was then analyzed using a UV spectrophotometer.

Table 7-8 displays the outcomes of the evaluation tests and dissolution test results. Calibration curve for diethylcarbamazine citrate by dissolution illustrated in figure 5, Spectrum overlay graph for dissolution by UV spectrophotometer illustrated in figure 6, overlay graph for the commercially available tablet illustrated in figure 7. Overlay graph depicting expired tablets is presented in figure 8.

Table 10: Evaluation test of diethylcarbamazine citrate

Evaluation test	Parameter	Result	
		Non-expired tablet	Expired tablet
Hardness test	Hardness	4kgcm^{-1}	2.5kgcm^{-1}
Disintegration test	Time	15 min	30 min
Dissolution test	%Drug release	98%	78.48%

Table 11: Table for dissolution calibration curve

Dissolution Time	Absorbance
10min	0.041
20min	0.050
30min	0.068
40min	0.077
50min	0.080
60min	0.090
70min	0.099

Fig,5: Calibration curve for diethylcarbamazine citrate by dissolution

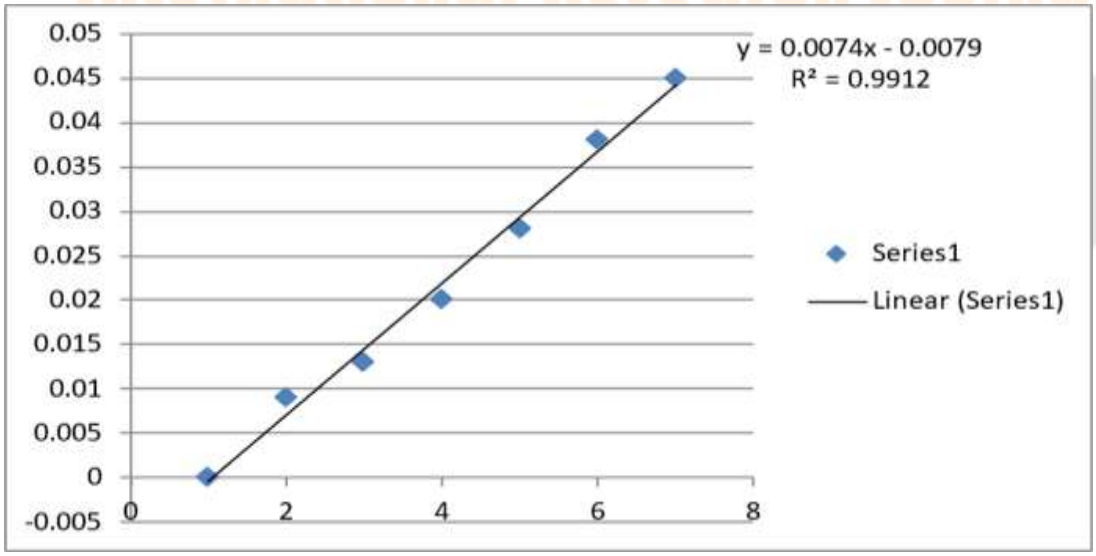
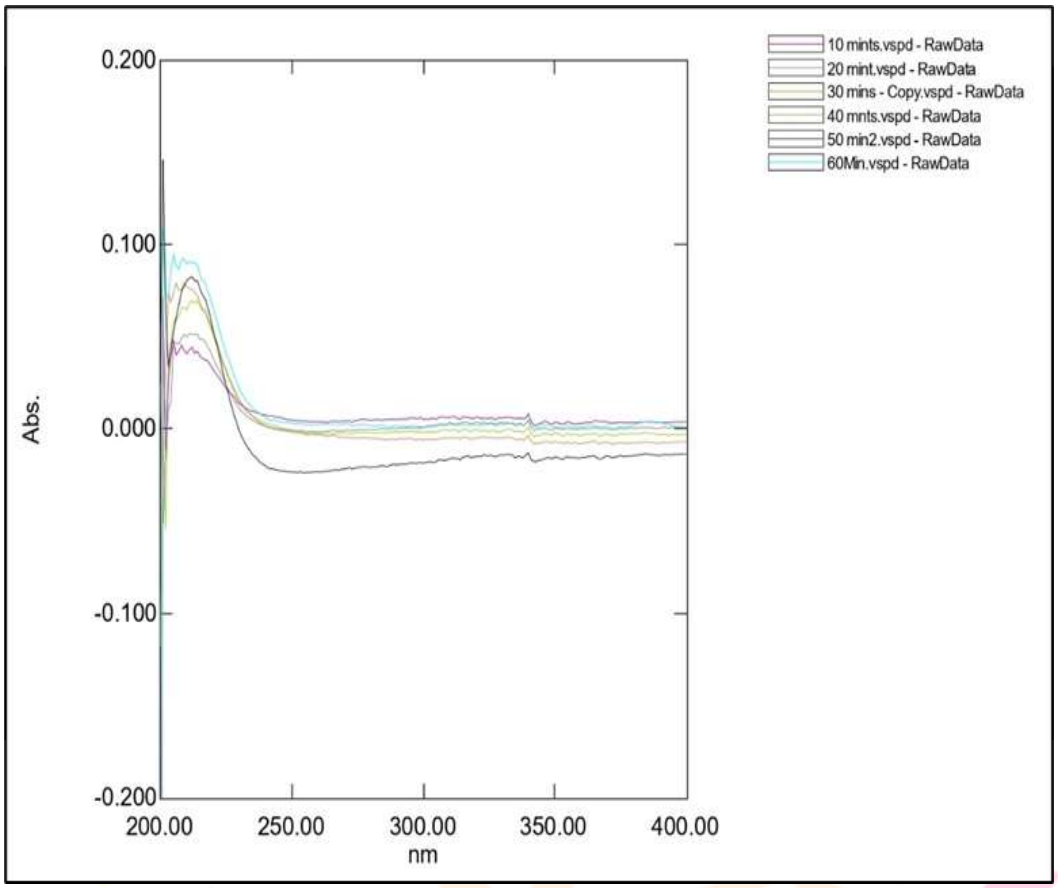


Fig.6: Spectrum overlay graph for dissolution by UV spectrophotometer



Formula for percent drug release from the tablet $\%Drug\ Release = Concentration \div$ Label Claim × 100

Table 12: Calculation table for marketed tablet

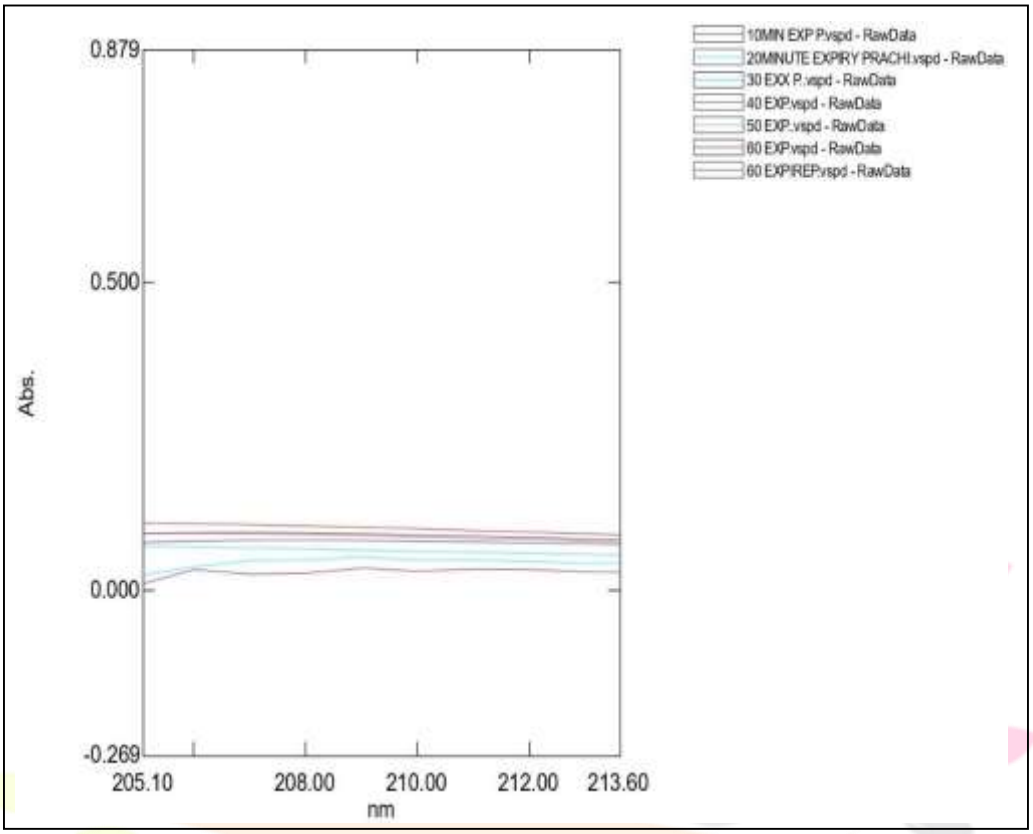
Time	Absorbance	Concentration (1in 10)	Concentration (10ug/ml)	Conc. in 900 media	Cumulative in 900 media	%Drug Release
10min	0.041	6.85	68.5	61650	61.65	41.1
20min	0.050	8.14	81.4	73260	73.26	48.84
30min	0.068	10.71	107.1	96390	96.39	64.2
40min	0.077	12	120	108000	108	72
50min	0.080	12.42	124.2	111780	111.78	74.4
60min	0.090	13.85	138.5	124650	124.65	82.8
70min	0.099	15.14	151.4	136260	136.26	90.6

Table 13: Calculation table for Expired tablet

Time	Absorbance	Concentration (1in 10)	Concentration (10ug/ml)	Conc. in900 media	Cumulative in 900 media	%Drug Release
10min	0.015	3.14	31.4	28260	28.26	18.84
20min	0.027	4.85	48.5	43650	43.65	29.1
30min	0.038	6.42	64.2	57780	57.78	38.52
40min	0.047	7.71	77.1	69390	69.39	46.26
50min	0.059	9.42	94.2	84780	84.78	56.52
60min	0.068	10.71	107.1	96390	96.39	64.26
70min	0.075	11.71	117.1	105390	105.39	70.26

Fig 7: Spectrum overlay graph for tablet (marketed) medicine

Fig 8: Spectrum overlay graph for expired medicine



Assay:

Forty pills of the test material were weighed and thereafter finely pulverized. A precisely measured amount of the powder, corresponding to around 1mg of diethylcarbamazine citrate, was put into a 100 ml volumetric flask and filled to 100 ml in order to achieve a concentration of 100 ppm. The volumetric flask was agitated by mechanical shaking for 5 minutes, then subjected to sonication for 10 minutes. It was then diluted to its original volume and processed using HPLC. The results of the assay research are displayed in table 9. Graph for assay of marketed tablet and expired tablets mentioned in fig.9-fig 10 resp.

$$\%\textbf{Purity for marketed tablet} = \frac{911493}{363920} \times \frac{2}{20} \times \frac{1}{10} \times \frac{20}{2} \times \frac{10}{1} \times \frac{150}{407} \times 100 = 98\%$$

$$\%\textbf{Purity for expired tablet} = \frac{487889}{223586} \times \frac{2}{20} \times \frac{1}{10} \times \frac{20}{2} \times \frac{10}{1} \times \frac{150}{407} \times 100 = 78.48\%$$

Results for assay of marketed and expired tablet:

Table 14: Calculation table for % purity of tablet

Sr.no.	Area	RSD	%PURITY
Marketed	458932	1.367%	98%
Expired	467889		78.48%

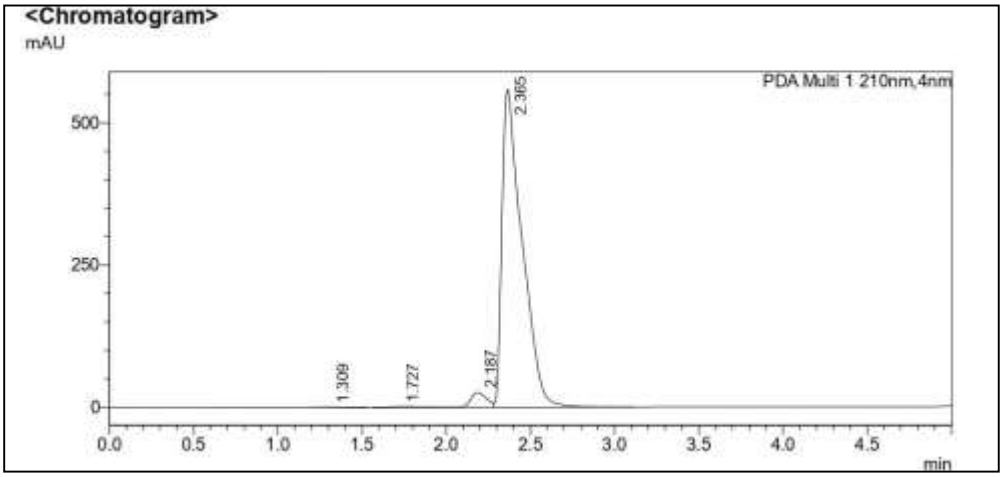


Fig 9: A typical chromatogram of diethylcarbamazine citrate for marketed tablets

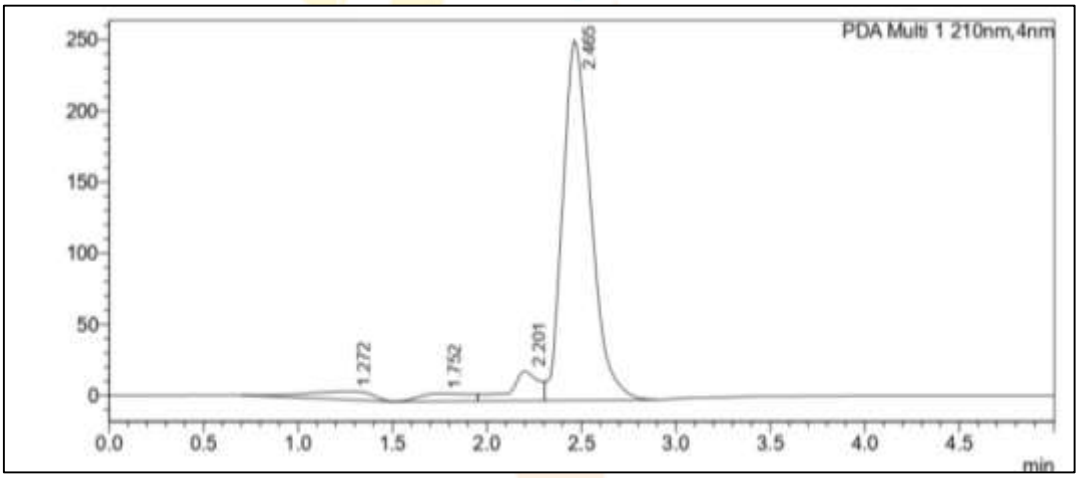


Fig 10: A typical chromatogram of diethylcarbamazine citrate for expired tablets

Conclusion:

Excellent linearity, sensitivity, accuracy, and precision may be achieved in estimating the tablet content utilizing the proposed RP-HPLC technique. The presented study demonstrates the development of a verified and specific stability-indicating RP-HPLC technique for diethylcarbamazine citrate, which has the capability to effectively separate the drug. The dissolving profiles of expired and non-expired diethylcarbamazine citrate tablets are differed, however this pilot study found no discernible physical distinctions between them. The efficacy of expired diethylcarbamazine citrate tablets is expected to be reduced due to their lower drug release compared to non-expired tablets. Furthermore, it was shown that the proportion of expired pills in the test was reduced compared to that of non-expired tablets.

The RP-HPLC method resolved DEC efficiently with a short retention time (2.365 min), aligning with prior studies. The hardness and disintegration time of expired tablets (2.5 kg/cm², 30 min) differed significantly from non-expired tablets (4 kg/cm², 15 min), indicating physical degradation. The created method has been verified in accordance with ICH requirements, and it has been advised that the method's specificity, linearity, precision, accuracy, and ruggedness be achieved. The tablet content can be estimated with good linearity, sensitivity, accuracy, and precision using the suggested RP-HPLC method. The dissolving profiles of expired and non-expired diethylcarbamazine citrate tablets varied, although there were no physical differences between them according to this pilot investigation. It is anticipated that expired diethylcarbamazine citrate tablets will be less effective since they released less energy than non-expired tablets. Additionally, it was discovered that the percentage assay of expired tablets was lower than that of non-expired tablets. The developed HPLC method can be used for the suitable quality control test for the estimation of diethylcarbamazine citrate (DEC) in bulk, marketed tablets and expired tablet.

Limitations:

The study did not identify degradation products or assess long-term stability under varied conditions. Future work should include forced degradation studies.

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